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CHEMOTHERAPY OF MALARIA

ANNUAL SUMMARY REPORT

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For the period of June 1, 1973 to May 31, 1974

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P. berghei blood-induced malaria infection in mice - based on mortality.

P. gallinaceum sporozoite-induced malaria infection - Aedes aegypti - chicks - based on mortality.

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Primary screen - quantitative evaluation of potential antimalarial activity.

Primary screen - to provide quantitative assessments of prophylactic values.

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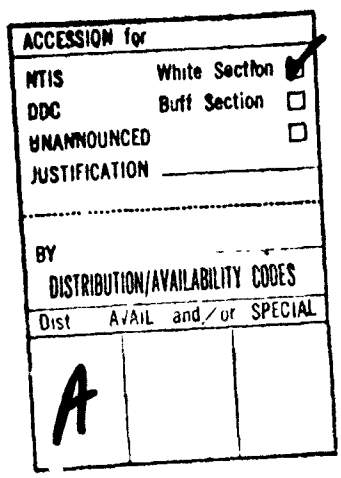
Foreword

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.



This antimalarial screening program was initiated after the discovery that the Plasmodium falciparum parasite chiefly responsible for the high incidence of malaria in Vietnam was resistant to drugs such as chloroquine, generally recognized since World War II as satisfactory antimalarial agents. The urgent need for the screening operation was further emphasized when the same resistant strain, and others, were encountered in different malaria centers in Asia and in South America.

A total of 236,137 compounds were tested from December 1, 1961, through May 31, 1974.

177,844 were screened in the period from December 1, 1961, through May 31, 1970, covered by Contract No. DA-49-193-MD-2218. Table I provides annual summaries of the number of compounds evaluated and the number of mice used in this period.

Continuing under Contract No. DADA 17-70-C-0100, activated on June 1, 1970, Tables II, III and IV record the number of compounds screened and the number of mice used for each contract year from June 1, 1970, through May 31, 1973.

Table V presents monthly summaries of the compounds tested and the mice used from June 1, 1973, through May 31, 1974.

The test system designed specifically for this operation is based on blood-induced Plasmodium berghei malaria infections in mice.* It is a relatively simple and fast procedure. Its assessments of antimalarial effects and host toxicity are reproducible and reliable. It has introduced a new efficient method for screening large numbers of candidate compounds that has been successfully used as a model in the development of other large scale screening operations.**

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* Designed, developed and operated by Dr. Leo Rane until his sudden death on June 21, 1973.

** (1) A screening operation using blood-induced P. gallinaceum infections in chicks.
(2) A screening operation using sporozoite-induced P. gallinaceum infections in chicks.
(3) A screening operation based on blood-induced Trypanosoma cruzi-desiense infections in mice.

All compounds evaluated were obtained from the Department of Medicinal Chemistry at the Walter Reed Army Institute of Research and included:

(1) compounds structurally related to chemicals of known value as antimalarial agents;

(2) compounds structurally unrelated to compounds known to have antimalarial activity;

(3) structural analogues of compounds found active in our test system and representing several novel chemical groups.

Our own breeding colony of ICR/HA Swiss mice has continued to supply the animals used in our tests.

Evaluations of activity have been based on the responses to candidate compounds by Plasmodium berghei malaria in mice as expressed in comparisons of the maximum survival time of treated malaria-infected animals and the survival time of untreated malaria-infected controls.

TABLE I

SUMMARY OF SCREENING LEVELS

DECEMBER, 1961 - MAY, 1970

	NUMBER OF COMPOUNDS	NUMBER OF MICE
DECEMBER, 1961 - MAY, 1964	6,915	250,000*
JUNE, 1964 - MAY, 1965	13,114	215,715
JUNE, 1965 - MAY, 1966	22,731	350,449
JUNE, 1966 - MAY, 1967	34,093	531,200
JUNE, 1967 - MAY, 1968	40,465	636,525
JUNE, 1968 - MAY, 1969	38,150	603,225
JUNE, 1969 - MAY, 1970	22,376	411,270
TOTAL	177,844	2,998,384

*Includes mice used in the development of the test.

TABLE II

MONTHLY SCREENING LEVELS

JUNE 1, 1970 - MAY 31, 1971

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
JUNE	2,130	35,925
JULY	1,059	19,140
AUGUST	692	14,310
SEPTEMBER	884	19,140
OCTOBER	1,073	19,140
NOVEMBER	1,264	23,925
DECEMBER	1,484	25,125
JANUARY	1,385	23,910
FEBRUARY	1,328	23,760
MARCH	2,330	39,045
APRIL	2,650	45,330
MAY	1,829	33,390
TOTAL	18,108	322,140

TABLE III

MONTHLY SCREENING LEVELS

JUNE 1, 1971 - MAY 31, 1972

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
JUNE	1,557	27,390
JULY	1,129	26,250
AUGUST	1,194	21,480
SEPTEMBER	1,069	19,080
OCTOBER	1,447	23,850
NOVEMBER	811	14,310
DECEMBER	795	1[illegible],310
JANUARY	1,035	19,080
FEBRUARY	1,215	20,265
MARCH	1,745	28,590
APRIL	1,439	23,820
MAY	1,438	23,820
TOTAL	14,874	262,245

TABLE IV

MONTHLY SCREENING LEVELS

JUNE 1, 1972 - MAY 31, 1973

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
JUNE	1,830	29,775
JULY	985	16,605
AUGUST	1,142	20,205
SEPTEMBER	969	16,620
OCTOBER	1,192	19,050
NOVEMBER	1,204	19,080
DECEMBER	959	15,240
JANUARY	1,248	19,485
FEBRUARY	1,154	18,090
MARCH	1,742	27,750
APRIL	944	15,270
MAY	907	14,280
TOTAL	14,276	231,450

TABLE V

MONTHLY SCREENING LEVELS

JUNE 1, 1973 - MAY 31, 1974

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
JUNE	923	14,284
JULY	1,000	15,480
AUGUST	451	7,275
SEPTEMBER	940	14,280
OCTOBER	957	14,550
NOVEMBER	1,020	15,525
DECEMBER	698	10,610
JANUARY	782	11,880
FEBRUARY	940	14,300
MARCH	1,145	17,850
APRIL	940	14,280
MAY	1,209	18,350
TOTAL	11,035	168,664

Using young ICR/HA Swiss mice and a standard inoculum of Plasmodium berghei, it has been possible to produce a consistently uniform disease fatal to 100% of untreated animals within 6 to 8 days.

Since an established disease is less responsive to treatment than a disease in the early stages of development, treatment is withheld deliberately until a fairly high degree of parasitemia is evident. Test compounds are administered parenterally in a single dose on the third day post-infection by which time a 10-15% parasitemia has developed.

To be classified as active, a compound must suppress the disease and produce an unquestionably significant increase, 100% or more, in the life span of the treated animals over that of the untreated controls.

The severity of the challenges set up in our test system enhances the reliability of our evaluations and the antimalarial potential of the compounds selected for intensive preclinical studies.

METHOD

ANIMAL HOSTS. The total supply of animals needed to screen candidate compounds has been obtained from our own breeding colony of ICR/HA Swiss mice. Test animals weigh from 18 to 20 grams, weight variations in any given experimental or control group being carefully limited to 2-3 grams. In any given test all animals are of a single sex and approximately the same age.

Animals on test are housed in metal-topped plastic cages, fed a standard laboratory diet and given water ad lib.

TEST PROCEDURE. Test animals receive an intraperitoneal injection of 0.5 ml. of a 1:100 dilution of heparinized heart's blood with a minimum of 90% parasitized cells, drawn from donor mice infected one week earlier with Plasmodium berghei. The donor strain is maintained by weekly passages in separate groups of mice inoculated with 0.5 ml. of a 1:50 dilution of heparinized heart's blood.

In order to check factors such as changes in the infectivity of our Plasmodium berghei strain or in the susceptibility of the host or to detect technical errors, a group of infected animals treated with pyrimethamine at dose levels known to produce definite increases in survival time is included in every experiment as a posit've control.

DRUG ADMINISTRATION. Test compounds are dissolved or suspended in peanut oil before they are administered.

Treatment consists of a single dose given subcutaneously 3 days post-infection. At the time of treatment a 10-15% parasitemia has developed. Although the disease is well established, it has not yet caused sufficient debility to affect an evaluation of the test compound's toxicity.

Deaths that occur before the 6th day, when untreated controls begin to die, are regarded as the result of a compound's toxic effects and not as the result of action by the infecting parasite.

In each experiment the compound on test is administered in graded doses. Increases in the dose levels of highly active compounds usually are followed by increases in the survival time of the treated mice.

If an active drug is toxic for the host, the toxicity of this compound may become a limiting factor to changes in dose levels.

Treated animals alive at the end of 60 days are considered as cured.

DRUG ACTIVITY. Acceptance of a drug as being sufficiently active for detailed studies is predicated on the margin between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED). A maximum tolerated dose is defined as the highest dose causing no more than one of five animals to die. The minimum effective dose is defined as the minimum dose increasing the life span of treated animals by 100% over the life span of untreated controls.

An increase of 100% in survival time is considered the minimum significantly effective response for a candidate compound.

Clearly inactive compounds are rejected after one test, borderline compounds after two tests. Active compounds are subjected to a dose-response curve so that the spread between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED) may be established.

COMPOUNDS WITH DEFINITE CHEMOTHERAPEUTIC ACTIVITY AGAINST PLASMODIUM BERGHEI IN MICE

Of the 18,108 compounds tested from June 1, 1970, through May 31, 1971, 805 demonstrated a degree of antimalarial activity sufficient to produce at least 100% increases in the survival time of treated Plasmodium

berghei infected mice.

Of the 14,276 compounds tested from June 1, 1972, through May 31, 1973, more than 771 demonstrated a degree of antimalarial activity sufficient to produce at least 100% increases in the survival time of treated Plasmodium berghei infected mice.

Of the 11,035 compounds tested from June 1, 1973, through May 31, 1974, about 394 demonstrated a degree of antimalarial activity sufficient to produce at least 100% increases in the survival time of treated Plasmodium berghei infected mice.

It is generally conceded that a screening procedure with sporozoite-induced Plasmodium gallinaceum malaria in chicks could be effective both as a primary screen of therapeutic and/or prophylactic activity or as a confirmatory test.

The development of such a procedure was undertaken primarily to assess prophylactic values of candidate compounds.

In its initial phase, under Contract No. DA-49-193-MD-2218, the progress of our study was completely dependent on weekly shipments of frozen infected material prepared by an outside and distant supplier.

Within a period of twelve months studies with approximately 12,000 chicks and 200 compounds indicated that our dependence on this routine limited our investigations and thwarted our efforts to design a sporozoite-induced avian test approaching the degree of uniformity and reproducibility of our blood-induced mouse test and our blood-induced chick test. We were convinced that our studies would continue to be hampered until we were able to prepare the necessary infected material on our own premises.

Under Contract No. DADA 17-70-C-0100, activated June 1, 1970, we were authorized to set up a mosquito rearing facility to provide the Aedes aegypti that we would require.

The sporozoite-induced avian malaria test that we have developed, like our blood-induced mouse test and our blood-induced chick test, is based on mortality, not on morbidity.

Modifications in methods of rearing and handling non-infected and infected mosquitoes were required to achieve this end.

These modifications, highly controlled, involved:

(a) nutritional requirements of larvae and pupae;
(b) more satisfactory methods of feeding and watering adult mosquitoes prior to and following blood meals;
(c) types of blood meals;
(d) methods for preparing a standard inoculum;
(e) avenues of drug administration and regimens.

Our results, obtained from studies with more than 133,355 chicks, indicate that our sporozoite-induced screen has met the standards demonstrated in our blood-induced screens.

Using chicks weighing 53-57 grams and a standard inoculum of sporozoites from Plasmodium gallinaceum infected Aedes aegypti mosquitoes, we have been able to produce a disease that is fatal to 100% of untreated controls within 7.0 - 10.6 days, an overall average of 8.46 days.

Prophylactic activity is assessed by comparing the maximum survival time of treated sporozoite-infected chicks and the survival time of untreated sporozoite-infected controls.

A compound is considered active if it has produced increases of at least 100% in the survival time of treated chicks over the survival time of untreated controls.

Acceptance of a compound's prophylactic activity is predicated on the margin between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED).

METHOD

TEST ANIMAL. White leghorn cockerels weighing 53-57 grams are used in these tests.

The birds, of fairly uniform stock, are purchased from local hatcheries, delivered to the laboratory when 1 day old and then maintained under standard conditions, including a non-medicated diet, until they are ready for a test.

TEST PROCEDURE. Chicks on test receive an intrajugular injection of 0.5 ml. of Plasmodium gallinaceum infected Aedes aegypti mosquitoes, ground whole and containing sporozoites.

The parasite strain is maintained in separate groups of chicks bitten by infected mosquitoes.

In order to check factors such as changes in the infectivity of the sporozoites or the Plasmodium gallinaceum strain, changes in the susceptibility of the host or simply to detect technical errors, a group of infected chicks treated with sulfadiazine at dose levels known to produce definite increases in survival time is included as a positive control in every experiment.

DRUG ADMINISTRATION. Treatment consists of a single dose administered subcutaneously or per os on the day of infection.

Compounds are dissolved or suspended in peanut oil before they are administered.

Each test is done with graded doses of the candidate compound, and increases in the dose levels of highly active compounds have generally been followed by increases in the survival time of the treated chicks.

If an active compound is toxic for the host, its toxicity becomes a limiting factor in changes of doses.

Deaths that occur within 5 days after infection and treatment are considered as deaths due to toxic effects of a test compound, not as the result of the infection introduced by the sporozoites from infected mosquitoes.

Chicks with survival periods of 30 days are recorded as "survivors"

DRUG ACTIVITY. An increase of 100% in survival time has been considered as the minimum significantly effective response to the prophylactic activity of a compound.

COMPOUNDS WITH PROPHYLACTIC ACTIVITY AGAINST PLASMODIUM GALLINACEUM MALARIA IN CHICKS. Routine testing began October 1, 1971. Of the 6,305 compounds tested from that day through January 22, 1974, 1,050 demonstrated a degree of activity that produced a minimum of 100% increase in the survival time of sporozoite-induced Plasmodium gallinaceum infections in chicks.

It has become increasingly evident that the range of therapeutic and/or prophylactic activity that can be assessed in sporozoite-induced P. gallinaceum malaria infected chicks as in blood-induced

P. gallinaceum malaria infected chicks is far more limited than the range demonstrated in blood-induced *P. berghei* malaria infected mice.

This test will be phased out at the end of this contract year.

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